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Alcohol, Habituation and the Patterning of Aggressive Responses in a Cichlid Fish[1]

HARMAN V. S. PEEKE, SHIRLEY C. PEEKE, HARRY H. AVIS AND GEORGE ELLMAN

Laboratory of Psychobiology, Department of Psychiatry, University of California San Francisco CA 94143

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PEEKE, H. V. S., S. C. PEEKE, H. H. AVIS, AND G. ELLMAN. *Alcohol, habituation and the patterning of aggressive responses in a cichlid fish.* PHARMAC. BIOCHEM. BEHAV. 3(6) 1031–1036, 1975. – Territorial cichlids were presented for 30 min with a conspecific male intruder (contained in a clear glass tube). Eight hr prior, 2 groups were administered alcohol (0.15 or 0.30 percent in the aquaria water). A third group served as a control. Three responses were recorded to allow analysis of topographic changes in behavior as well as changes in absolute levels. During the habituation phase, the normal group showed a sequence of long displays, followed by shorter ones as the frequency of attacks increased. The occurrence of threat which gradually gives way to attack is characteristic of the agonistic behavior of this species. In contrast to the controls, the 0.15 percent group was hyperaggressive, while the 0.30 percent group was hypoaggressive. Furthermore, the patterning of responses were abnormal. The 0.15 percent group gave abbreviated threats and more attacks (interpreted as a tendency to attack without warning); whereas, the 0.30 percent group gave many long threat displays, but few attacks. A stimulus specificity test provided strong evidence that the waning found during the initial phase was habituation.

Habituation Aggression Alcohol Fish Cichlasoma Nigrofasciatum Response Topography Stimulus Specificity

THE present study was designed to investigate the effects of alcohol on the elicitation and habituation of aggression in territorial male convict cichlids. There are a number of advantages to the use of this species as a model for the effect of alcohol on aggression. A foundation of data has been provided by the use of lower vertebrates (especially fish and birds) upon which theories of aggression, territoriality and the maintenance of interpersonal space have been built. The extrapolation of these theories to human behavior (necessitated by a proscription against such research with humans) has been done with varying degrees of care and sophistication to yield, on the one hand, simplistic and probably erroneous conceptions of human aggression, but on a more sophisticated level, guidelines and hypotheses for future research.

The aggressive and territorial behavior of the convict cichlid has been studied extensively in the wild [8] and in the laboratory [6, 11, 21]. The time course of territorial establishment, breeding and raising of fry are well documented as are the respective roles of males and females at each phase. There is substantial evidence that a solitary male (the preparation used in the present study) will remain territorial for long periods of time, thus providing a relatively constant state of aggression upon which drug effects may be superimposed.

The drug treated convict cichlid also has advantages as a psychopharmacological tool [9]. If the drug is water soluble it is easily administered by adding it directly to the aquarium water and thus the maintenance of a steady blood alcohol level (BAL) is accomplished by maintaining a given concentration of alcohol in the aquarium. It should be noted that while the level of blood alcohol is proportional to aquarium concentration, the specific level is species-specific. Uptake studies for the convict cichlid have shown that it will maintain an asymptotic BAL of 65 percent of the aquarium concentration after 6 hr [10] whereas the goldfish maintains an asymptotic BAL of 85 percent after a similar length of time [17]. Also, like other species of fish, this preparation has numerous neurological similarities to mammals [5].

Previous research on changes in levels of aggression in fish as a result of alcohol administration has shown that a moderate dose of alcohol enhances aggressiveness whereas a much higher dose inhibits the behavior [16,15]. Since uptake data was not provided for the species used (Siamese fighting fish) in these two studies, dose level can not be determined. However, similar results were found by Peeke, *et al.* [10] where a very low dose (0.07 percent alcohol per vol of aquarium water) had essentially no effect; a moderate dose (0.18 percent) increased the frequency and duration of aggressive display and frequency of biting; while a third dose (0.33 percent) inhibited all 3 measures, producing a hypoaggressive but otherwise normal fish.

Since previous research has shown that various doses

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change initial responsiveness to an aggression-eliciting stimulus, it was reasoned that the course of habituation to such stimuli might also be changed by alcohol. Habituation, the waning of response to a repeated or constant stimulus, is perhaps a basic form of adaptive modification while being also one of the least studied learning phenomena. Recently we have attempted (see also [1,4]) to relate habituation to the reduction of aggressive encounters between adjacently territorial conspecifics. There is now evidence that habituation of aggressive behavior in territorial fish is persistent over time and very stimulus specific; all are criteria of a process that serves to lower aggression between neighbors, but maintain fighting readiness against intruders [12]. Field studies of birds suggest that a similar phenomenon operates in their spacing behavior.

There are several changes in the course of habituation that might occur as a result of alcohol. Habituation might take place at the normal rate (although from an altered starting point), at an altered rate (either faster or slower) or might not take place at all. An additional possibility would be specific effects upon the patterning of the sequence of the various aggression behaviors. Such an alteration in the topography of behavior would be particularly important since some behaviors serve as signal functions (threats or warnings) while other behaviors inflict injury. Several measures of the aggressive sequences of behavior are reported here as we feel that changes in the topography of behavior are as important and informative as changes in absolute level or intensity.

METHOD

Animals and Apparatus

Twenty-one adult male convict cichlids (*Cichlasoma nigrofasciatum*) were placed individually in glass aquaria (90 × 30 × 30 cm) containing a ceramic pot lying 10 cm from one end of the tank on a substrate of coarse gravel. The pot served as a refuge and could be considered the center of a territory. Air-driven filters maintained filtration and circulation, but were disconnected when the alcohol was introduced into the tanks for the duration of the experiment. Water temperature was maintained at 25°C. (±2°). Fish were fed live brine shrimp and dried food daily and were allowed to acclimatize to the situation for 5 days before the experiment began.

Procedure

Three groups (n = 7 in each) were used: 0.0, 0.15 and 0.30 percent concentration of ethanol in the water. Based on ethanol uptake studies using similar concentrations [10], the level of ethanol in the blood of the fish was expected to reach 65 percent of the water concentration after a period of 6 hr.

These doses were selected to provide additional points to supplement previously obtained data concerned with the function relating alcohol concentration to aggression [10]. The alcohol was introduced with stirring at 0800 in quantities to conform to one of the doses. Water samples were taken after completion of the procedure (7 hr) to confirm alcohol concentrations in the tanks.

Six hours after introduction of the ethanol, the resident fish was presented with a live, male conspecific (confined to a clear glass tube, 100 mm dia., filled with water) at a spot 200 mm from the entrance to the ceramic pot. The intruding stimulus was left in place for 30 min (habituation phase) and then removed. After a 5 min period, a different stimulus fish was presented in the same manner for 2.5 min (stimulus specificity test phase). An increase in response to this stimulus following initial decrement in response would indicate that the fish was still capable of vigorous response and would control for fatigue and sensory adaptation, two general criteria for demonstrating that a decrement in response is truly habituation.

The experimenter was situated in front of the aquarium with a hand console containing buttons which, when pushed, registered frequency and duration of various responses on an event recorder. The responses recorded were: (1) frequency of displays, where a display was counted each time the fish extended gill covers and branchiostegal membranes; (2) duration of each display; and (3) frequency of bites at the glass tube containing the stimulus fish. Three measures were analyzed separately and a fourth, average display duration, was calculated from the data on total display duration and frequency of display. This data yielded a measure of duration of the average threat display at each time over the course of the experiment.

RESULTS

This study had two primary concerns. The first was the effect of the 3 doses of alcohol on the overall level of responding in each measure. Thus, the first set of analyses compare the 3 groups of animals on each of the 3 measures. The second concern was whether a given dose of alcohol affected the topography of the responses, that is, the interrelations of the responses with respect to ease of elicitation, rate of habituation, degree of recovery, etc. Thus, the second set of analyses provide a more detailed description of the effects of each dose of alcohol on the initial level and time course of each of the measures. Each measure was graphed for each drug condition over the 30 min of habituation and the 2.5 min of the stimulus specificity test in Fig. 1.

The data of the 30 min of the habituation phase were divided into twelve 2.5 min blocks.

Overall Effect of Dose on Elicitation and Habituation of Aggressive Behaviors

Display frequency. A blocks by groups analysis of variance was performed which revealed a significant time blocks effect, $F(11,198) = 5.58$, $p<0.01$. The blocks effect reflects an overall downward trend in response. Examination of the group means reveal that the 0.15 percent group gave the most displays ($\bar{X} = 50.00$) followed by the 0 percent ($\bar{X} = 27.9$) and the 0.30 percent group ($\bar{X} = 12.0$). T-tests indicated that all groups differed significantly from each other ($t = 5.36$, $p<0.01$ for 0.15 vs 0 percent; $t = 6.89$, $p<0.01$ for 0 vs 0.30 percent; $df = 12$ all tests).

Average display duration. A blocks by groups analysis of variance demonstrated a reliable blocks effect (reflecting an overall diminution in duration of response over time, $F(11,198) = 8.83$, $p<0.01$) and a significant interaction between groups and blocks $F(22,198) = 2.32$, $p<0.01$. This effect appeared to be due to absence of waning in the 0.15 percent group. To test this supposition, the first 3 time blocks were pooled and compared with the pooled score for the last 3 blocks by *t*-tests (for correlated means) done separately for each group. The normal group showed a

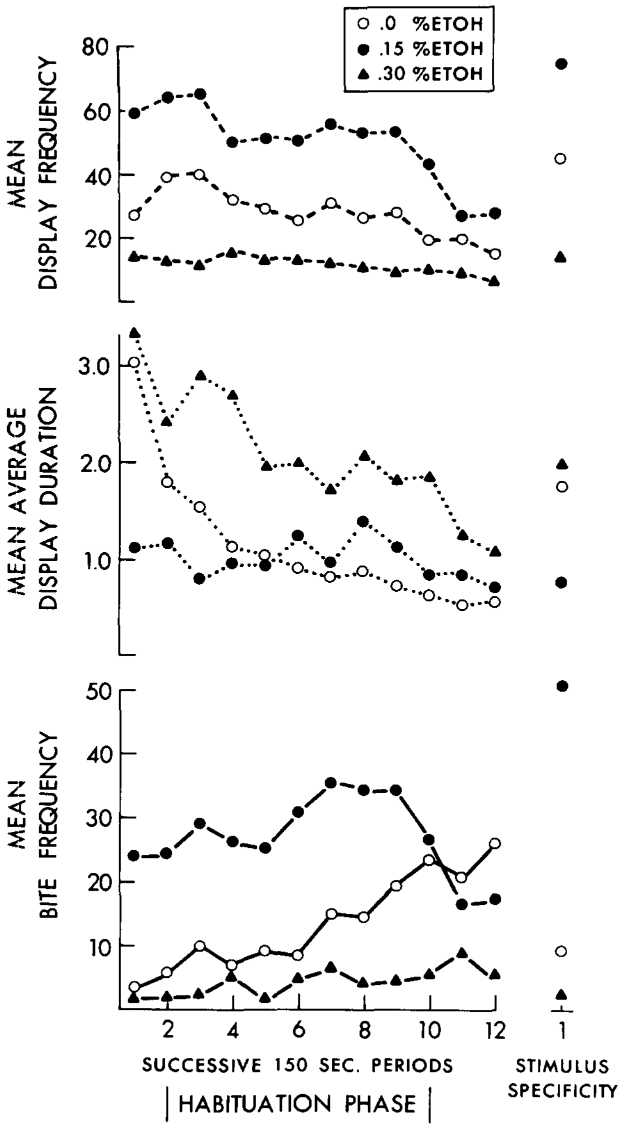

FIG. 1. Effects of 2 alcohol dosages and control on 3 indicies of aggression over the 12 2½ min blocks of the habituation phase and the 2½ min stimulus specificity test.

significant drop (t = 3.15, p<0.025, df = 6) as did the 0.30 percent group (t = 2.75, p<0.05, df = 6). The 0.15 percent group did not diminish significantly between first and last three blocks.

Bites. A blocks by groups analysis of variance for this measure yielded a significant groups effect, $F(2,18) = 4.87$, p<0.05, a significant blocks effect, $F(11,198) = 1.88$, p<0.05, and a significant interaction, $F(22,198) = 1.73$, p<0.05. Examination of the group means indicated that, as with the display frequency measure, the 0.15 percent group was most responsive ($\bar{X}$ = 46.7) with the 0 percent group intermediate ($\bar{X}$ = 27.3) and the 0.30 percent group least responsive ($\bar{X}$ = 13.72). T-tests indicated that each group differed significantly from each other group (t = 4.80, p<0.01 for 0.15 vs 0 percent, t = 4.01, p<0.01 for 0 vs 0.30 percent, df = 12 all tests). The significant overall blocks effect reflects a general upward trend in responding, but the significant interaction suggests that there must also be some difference in trend between groups. To test this supposition, a t-test (correlated means) was performed between the pooled first 3 and pooled last 3 points for each group. The 0 percent group showed a reliable increase from the start to the finish of the period (t = 2.40, p<0.05, df = 6). Neither of the alcohol groups showed a reliable change over the course of the habituation phase.

Overall Effect of Dose on Stimulus Specificity

With the presentation of a new stimulus after the habituation phase, it was expected that the various responses would return to their prehabituation levels or at least approach those levels. Tests were performed comparing the last block of the habituation phase with the stimulus specificity test.

Display frequency. An analysis of variance yielded a significant increase between the two blocks, $F(1,18) = 18.37$, p<0.01, as well as a significant difference between groups, $F(2,18) = 3.38$, p<0.01. The groups effect reflects differences between groups in overall response level and does not add new information. The upward trend of the blocks effect indicates that all 3 groups showed recovery of response, although to differing degrees.

Average display duration. There was no groups effect, but a reliable blocks effect, $F(1,18) = 13.75$, p<0.01, indicating recovery of response during stimulus specificity testing.

Bites. This measure yielded results somewhat different from the others. There was a significant groups effect, $F(2,18) = 4.91$, p<0.05, and a significant interaction, $F(2,18) = 10.80$, p<0.01. Inspection of Fig. 1 shows that the 0 and 0.30 percent groups show small decrease, and the 0.15 percent group showed a large increase. The decreases for the 0 and 0.30 percent groups returned them to their initial level of responding. To return responding of the 0.15 percent group to its initial level would have required an increase, and this is what was found; however, the increase that occurred far exceeded the initial and also the peak levels of responding. Inspection of scores for individual animals indicated that 3 of 7 animals in that group responded to the stimulus specificity test by an increase of 1.5 to 2 times their individual peak level for the habituation phase. Three other animals responded at slightly below peak level and one animal responded far below peak level. Thus, 42 percent of the animals were hyperaggressive as a result of the stimulus specificity test suggesting that the initial exposure may have sensitized those animals to later exposure to an intruding stimulus fish.

Comparison with the earlier study. An earlier study whose purpose was to study the initial response to the stimulus rather than habituation, used an identical procedure for 8 min of stimulus exposure with doses of 0, 0.07, 0.18 and 0.33 percent alcohol. Comparison of the first 8 min of behavior for the groups of the present study provide complementary points in the 8 min dose-response function reported previously [10]. Figure 2 presents these combined data expressed as a percentage of the response of the 0 percent alcohol groups. As the figure shows, the present study is quite consistent with the earlier results and serves as a replication of the 1973 study.

Effect of Dose on Response Topography – Habituation Phase

Zero percent alcohol group. The long-term waxing and

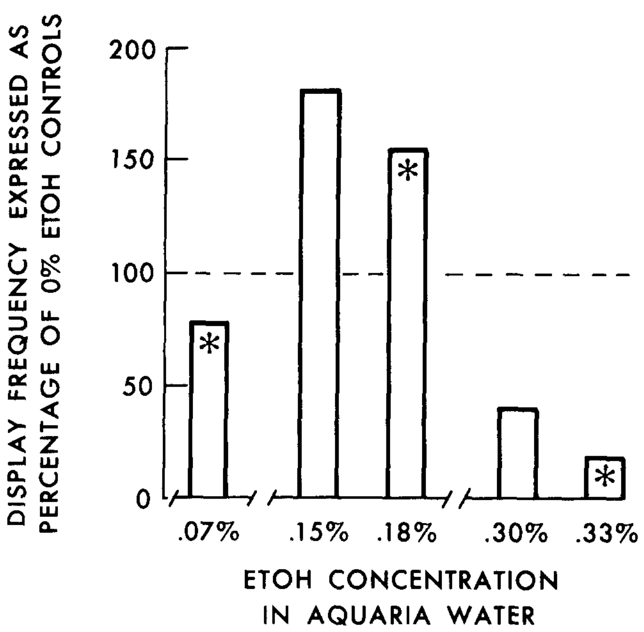

FIG. 2. Dose-response effects on 1 index of aggression (display frequency) combining data for the first 8 min of the present study and the comparable data from a previous study (7) using the same method (asteriks).

waning of aggressive response to a live conspecific stimulus have been described previously [4, 9, 11]. Initially, the interactions are characterized by frequent, long duration threat displays which gradually wane in frequency and duration and give way to attack in the form of biting. The attack also wanes if the intruder is not driven from the territory. The results observed here of the nondrug group (a decrease in average display duration with an increase in biting over the 30 min, Fig. 3) are consistent with the previous findings and are characteristic of each individual fish in the group as well. This behavioral sequence is, therefore, no artifact of grouping animals for means. The functional utility of this sequence is clear, the threat (display) precedes the appearance of violent behavior (bites), thus providing a mechanism whereby intrusions and boundary disputes can be abbrogated without violence, or at least without unsignalled violence.

Moderate (0.15 percent) alcohol group. Figure 4 illustrates a very different topography of the 3 responses over the course of the experiment. Instead of a picture of initially frequent long duration threats, decreasing over time as biting gradually increased, this group showed an initial frequency of displays nearly twice that of the nondrugged group and still increased in frequency over the first 20 min of the experiment. This topography is characteristic of a hyperaggressive fish, emitting short displays and bites at a high rate with no preceding period of long duration threats. Such a fish is responding to the intruding conspecific with immediate violent behavior with little or no warning.

High (0.30 percent) alcohol group. This group presents a picture (Fig. 5) very nearly the opposite of the moderate alcohol group (and can best be characterized as hypoaggressive relative to the normal group). The displays were less frequent (half as frequent as normals), although longer in

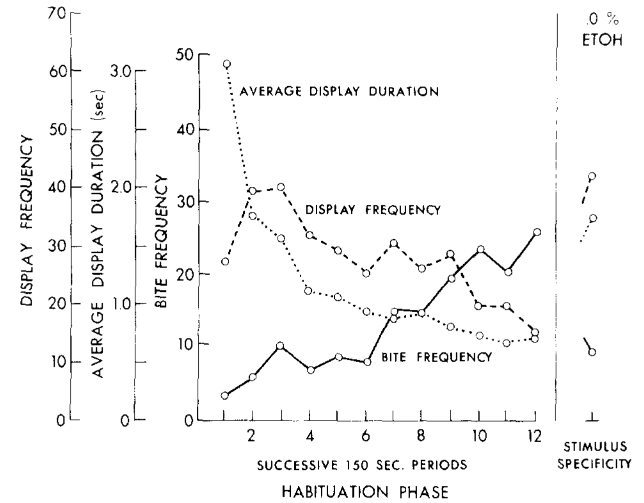

FIG. 3. Normal (no alcohol) group demonstrating the interaction (topography) of the 3 indicies of aggression over the 12 2½ min blocks (habituation phase) and the 2½ min stimulus specificity test.

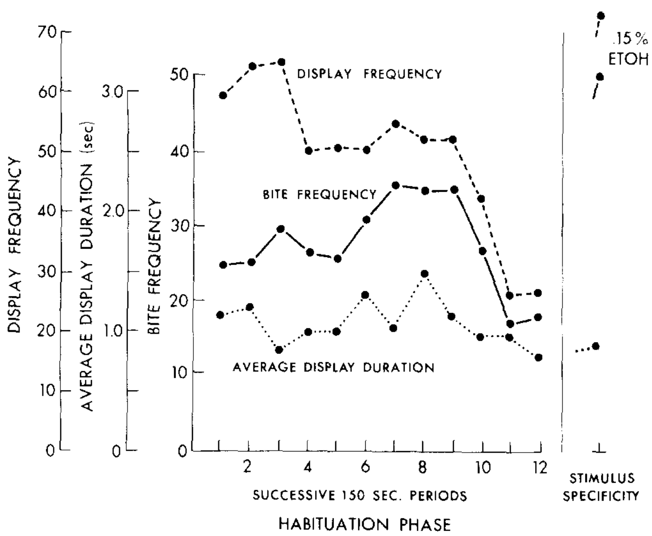

Fig. 4. 0.15 percent alcohol water concentration group demonstrating the interaction (topography) of the 3 indices of aggression over the 12 2½ min blocks of the habituation phase and the 2½ min stimulus specificity test.

duration. Bites showed no significant change from a near zero rate. If our supposition is correct that long duration threat displays are characteristic of an early stage in an aggressive encounter, then the high dose group would appear to be retarded in the progress of the sequence, maintaining long duration displays much longer than normal, but emitting them at a lower frequency than normal. Fish in this group gave no outward appearance of either disequilibrium or locomotor difficulty. Indeed, they were indistinguishable from the other groups in this regard.

Effect of Dose on Response Topography – Stimulus Specificity

If the change in behavior observed in response to one

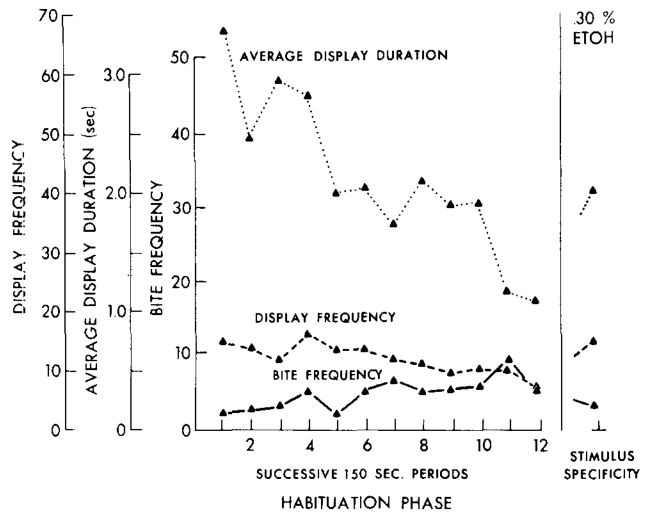

FIG. 5. 0.30 percent alcohol water concentration group demonstrating interaction (topography) of the 3 indicies of aggression over 12 2½ min blocks of the habituation phase and the 2½ min stimulus specificity test.

stimulus is specific to that stimulus, we would expect that a new stimulus would elicit a response comparable to that elicited by the original stimulus. Prior studies [13] showed that presentation of a novel stimulus fish in a new location resulted in complete recovery of responsiveness; presentation of the original stimulus fish in a new location or a new fish in the old location resulted in intermediate levels of recovery.

In the present study, the stimulus specificity test consisted of presenting a new stimulus fish in the old location and, on the basis of the study mentioned above [13] we would expect responding to revert in the direction of the initial level for each response. As Figs. 3, 4 and 5 show, that is what was found in all groups. Display frequency and average duration increased to near the original level while bites reduced in frequency which represents a return to initial stage (which consisted of a low level of biting).

The reaction of the moderate (0.15 percent) alcohol group to the novel stimulus fish was characterized by a very large increase in bites coupled with many very short displays. Rather than returning to the original level of responding as the non-alcohol group did, this group reacted with an exaggerated aggressive response to the novel intruder. The high (0.30 percent) alcohol group, on the other hand, showed less pronounced stimulus specificity than the other groups, probably due to the depressed level of responding in this group in general.

DISCUSSION

The results of this study leave little doubt that alcohol has profound effects on the elicitation and habituation of aggression in the convict cichlid. The effects are not only quantitative, but also qualitative when one considers the interrelationships of the various measures of aggression studied.

The moderate dose failed to produce the long threat displays characteristic of the normal fish before he delivered an attack. We conclude that such a fish would, in a face to face encounter with an unintoxicated rival, fail to provide the accustomed threat sequence before attacking and then attack with above normal frequency of bites. The result might well be that the unintoxicated antagonist would be defeated and driven off, or, in a confined space, severely injured or killed. On the other hand, the higher dose group showed hypoaggression, characterized by more long threats and very few bites even far along during the experimental period when the normal group had begun to shorten their displays and had commenced biting. It is likely that such a fish would be at a disadvantage in an agonistic encounter.

The fact that a moderate dose of alcohol produces one effect and a higher, but non-debilitating dose produces an opposite effect, with the normal group in between, would rule out such a simplistic explanation as that of alcohol being a general inhibitor. The biphasic action of alcohol has also been reported for certain human perceptual functions ([19] pp. 288–290 [22]) and for some types of human performance ([4] pp. 336–344 [18]). But the effect is not found for all tasks nor for all response systems and opposite effects may be found in different systems as when EEG slowing was observed concomitant with improved cognitive performance [2]. In human studies, the physiological state as well as the stressfulness of the experiment tend to modify the effects of a given dose ([20] p. 245 [3]). While it would be convenient to be able to speak of a single unidirectional effect of alcohol on aggressive behavior, the delineation of a dose-response function as complex as that observed in this study may be of greater benefit in explaining the many diverse effects that alcohol has on behavior.

REFERENCES

1. Assem, J. van den and J. den Molen. Waning of the aggressive response in the three-spined stickleback upon constant exposure to a conspecific. 1. A preliminary analysis of the phenomenon. *Behaviour* **34:** 286–324, 1969.
2. Doctor, R. F., P. Naitoh and J. C. Smith. Electroencephalographic changes and vigilance behavior during experimentally induced intoxication with alcoholic subjects. *Psychosom. Med.* **28:** 605, 1966.
3. Frankenhaeuser, M., E. Dunne, H. Bjurstrom and U. Lundberg. Counteracting depressant effects of alcohol by psychological stress. *Psychopharmacologia* **38:** 271–278, 1974.
4. Gallagher, J. E., M. J. Herz and H. V. S. Peeke. Habituation of aggression: the effects of visual social stimuli on behavior between adjacently territorial convict cichlids (*Cichlasoma nigrofasciatum*). *Behav. Biol.* 7: 359–368, 1972.
5. Ingle, D. J. The use of the fish in neuropsychology. *Perspect. Biol. Med.* **8:** 241–260, 1965.
6. Jellinek, E. M. and R. A. McFarland. Analysis of psychological experiments on the effects of alcohol. *Q. Jl Stud. Alcohol* **1:** 272–371, 1940.
7. Lorenz, K. Z. Ritualized fighting. In: *The Natural History of Aggression,* edited by J. D. Carthy and F. J. Eblins. New York: Academic Press, 1964, p. 39.
8. Meral, G. H. The adaptive significance of territoriality in New World cichlidae. Unpublished doctoral dissertation, University of California, 1973.
9. Peeke, H. V. S. and H. H. Avis. Studies of behavioral and pharmacological inhibition of territorial aggression in fish. In: *Origins and Determinants of Aggression,* edited by J. DeWitt and W. Hartup, Mouton, 1975.

10. Peeke, H. V. S., G. Ellman and M. J. Herz. Dose dependent alcohol effects on the aggressive behavior of the convict cichlid *(Cichlasoma nigrofasciatum). Behav. Biol.* **8:** 115–122, 1973.
11. Peeke, H. V. S., M. J. Herz and J. E. Gallagher. Changes in aggressive behavior in adjacently territorial convict cichlids. *(Cichlasoma nigrofasciatum):* The role of habituation. *Behaviour* **40:** 43–54, 1971.
12. Peeke, H. V. S. and S. C. Peeke. Habituation in fish with special reference to intraspecific aggressive behavior. In: *Habituation, Vol 1, Behavioral Studies,* edited by H. V. S. Peeke and M. J. Herz. New York: Academic Press, 1973, p. 59.
13. Peeke, H. V. S. and A. Veno. Stimulus specificity of habituated aggression in the three-spined stickleback (*Gasterosteus aculeatus*). *Behav. Biol.* **8:** 427–432, 1973.
14. Petrinovich, L. F. and H. V. S. Peeke. Habituation of territorial song in the white-crowned sparrow *(Zonotrichia leucophrys). Behav. Biol.* 8: 742–748, 1973.
15. Raynes, A. E. and R. S. Ryback. Effects of alcohol and congeners on aggressive response in *Betta splendens. Q. Jl Stud. Alcohol* **5:** 130–135, 1970.
16. Raynes, A. E., R. S. Ryback and D. J. Ingle. The effect of alcohol on aggression in *Betta splendens. Communs Behav. Biol.* **2:** 141–146, 1968.
17. Ryback, R., B. Percarpio and J. Vitale. Equilibration and metabolism of ethanol in the goldfish. *Nature* **222:** 1068-1070, 1969.
18. Tong, J. E., V. J. Knott, D. J. McGraw and G. Leigh. Alcohol, visual discrimination and heart rate. *Q. Jl Stud. Alcohol* **35:** 1003–1022, 1974.
19. Wallgren, H. and H. Barry, III. *Actions of Alcohol.* Vol. 1, Elsevier: New York, 1970.
20. Wilder, J. *Stimulus and Response: The Law of Initial Value.* John Wright and Sons, Bristol, 1967.
21. Williams, N. J. On the ontogeny of behavior of the cichlid fish, *Cichlasoma Nigrofasciatum.* Unpublished doctoral dissertation, University of Gronigen, 1972.
22. Zirkle, G. A., O. B. McAtee, P. D. King and R. Van Dyke. Meprobamate and small amounts of alcohol: Effects on human ability, coordination and judgement. *J. Am. Med. Ass.* **173:** 1823–1825, 1960.